NTP
National Toxicology Program

Concept Review:
The Mouse Methylome Project

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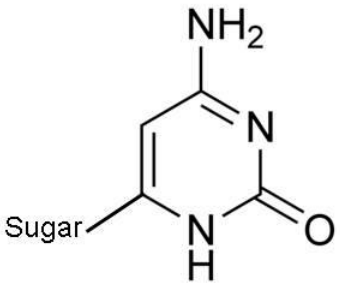
Outline

- Introduction to the methylome
- Proposed study
  - Animal study
  - Sample analysis
  - Data analysis
  - Significance and expected outcome

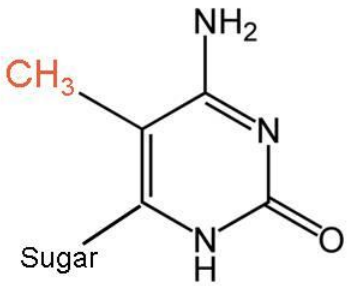
The Methylome

- Comprises the positions of all methylated cytosines in the genome
- It is one component of the epigenome, which controls non-genetic inheritance of phenotype

Cytosine vs. 5-methylcytosine



cytosine



5-methylcytosine

Biological Role of the Methylome

- X chromosome inactivation
- Imprinting
- Embryogenesis
- Gametogenesis
- Establishment of cell type specific patterns of gene expression
- Silencing of repetitive DNA elements in healthy and diseased cells

Disease Role of the Methylome

- Cancer
  - Increased methylation of tumor suppressors genes
  - Global demethylation leads to chromosome instability
- Neurodevelopmental disorders
- Neurodegenerative and neurological disease
- Autoimmune disease

Variation of the Methylome

- The methylome varies as function of
  - Genetics
  - Sex
  - Age
  - Nutritional status
  - Chemical exposure
  - Disease state
  - **Evidence suggests methylome variation contributes significantly disease susceptibility**
  - **To date, no one has addressed methylome variation on a high resolution, genome-wide scale**

We propose to create of a high resolution methylome map and then to determine how it varies as a function of genetic background, sex, and parental inheritance

This will contribute to a basic understanding of how the methylome influences disease susceptibility and will facilitate the incorporation of methylome assessment into toxicity testing

Model System

- C57BL/6N, C3H/HeN and B6C3F1 liver
- Liver is approximately 80-90% hepatocytes making it relatively homogenous
- B6C3F1 has been used for 30 years in the NTP bioassay
- Common target organ in cancer bioassays
- Spontaneous incidence rate of liver tumors
  - C57BL/6N <10%; C3H/HeN =100%; B6C3F1 = 30-50%
  - higher rates in males
- Increased liver tumor incidence often occurs following treatment with non-genotoxic chemicals which has been associated with alterations in the methylome

Specific Aims

- In depth, genome-wide map of the liver methylome of C3H/HeN, C57BL/6N, and B6C3F1/N mice
- Identify regions of the liver methylome that vary within and between strains
- Identify regions of the liver methylome that vary between sexes
- Identify heritable regions of the methylome and how the heritability of these regions vary
- Correlate DNA methylation patterns with the transcriptome at a quantitative (expression level) and qualitative (splicing) level

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Sample Analysis - Technologies

- Next Generation Genome Sequencing
  - Will provide sequence of the mouse genomes to allow for subsequent mapping of BIS-Seq, ChIP-Seq, and RNA-Seq reads
- Next Generation Sequencing of Bisulfite treated DNA (BIS-Seq)
  - Will provide a high resolution map of the methylome
- Next Generation Sequencing of affinity-purified of methylated DNA (ChIP-SEQ)
  - Will provide a lower resolution map of the methylome
  - Needed because future studies of NTP FFPE samples will use this technology
    - Must understand how it compares with BIS-Seq
- Next Generation Sequencing of the transcriptome (RNA-Seq)
  - Detailed map of RNA expression

Sample Analysis (1)

- Study has been broken into phases due to the resource intensive nature of the project
- Phase 1
  - Focused on one quartet: C57BL/6N female, C3H/HeN male, B6C3F1 male and female
  - Genome sequencing of C57BL/6N female and C3H/HeN male
  - Bisulfite sequencing (BIS-Seq) of all 4 mice
  - Targeted methylome re-sequencing of specific sites (ChIP-Seq)
  - Whole transcriptome expression profiles by RNA-Seq of all 4 mice

Sample Analysis (2)

- Phase 2 – C3H/HeN female, C57BL/6N male, C3B6F1 male and female
  - Bisulfite sequencing (BIS-Seq) of all 4 mice
  - Targeted methylome re-sequencing of the specific sites (ChIP-Seq)
  - Whole transcriptome expression profiles by RNA-Seq of all 4 mice
- Phase 3
  - Additional replicates and targeted resequencing as necessary

Data Analysis (1)

- Bioinformatic and statistical methods are under development
- Determine and catalog the genome wide cytosine methylation patterns
- Determine intra- and inter strain variation in the methylome
  - Identify differentially methylated sites within females and males of both parental strains (B6 and C3) and their F1 hybrid
  - Identify differentially methylated sites between the parental strains (B6 and C3) and their F1 hybrid

Data Analysis (2)

- Determine sexual dimorphisms in DNA methylation patterns
- Correlate local methylation patterns in the genome with quantitative and qualitative variation in the transcriptome
- Identify heritable regions of the methylome
  - Compare the methylome of each of the parental B6 females to their B6C3F1/N female offspring and the C3 males to their B6C3F1/N males offspring.
  - For the reciprocal outcross, compare the methylome of each of the parental C3 females to their C3B6F1/N female offspring and the B6 males to their C3B6F1/N male offspring to determine effects of germline transmission of imprinted genes (cytosine methylation variation in known imprinted genes).

Expected Outcomes

- A definitive map of the differentiated liver methylome and transcriptome
- A definitive map of inherited genome methylation patterns (imprinting)
- Know the regions of the methylome that vary in genetically identical individuals
- Know the regions of the methylome that vary as a function of genetics and sex
- Know performance metrics of 2 different methods for evaluating the methylome

Significance

- Facilitate the identification of chemicals that perturb the methylome during different life stages
- Assist in the identification of methylome-based biomarkers that are predictive of (hepato)carcinogenic hazard
- Identify regions of the methylome that are particularly susceptible to environmental influence
- Assist in understanding the basis of individual susceptibility to disease

Future Directions and Goals

- Extend the approach to other collected tissues and to other inbred strains, if warranted
- Develop tools for methylome analysis of NTP FFPE archived tissues and archived human tissues
  - Identify loci that are sensitive to chemical-induced methylation changes
  - Identify differentially methylated loci that are predictive of individual susceptibility to liver cancer

NTP/DIR Mouse Methylome Collaboration

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